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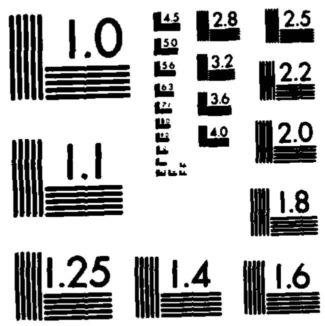

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AD-A143 960

FUNCTIONAL ASSESSMENT OF LASER IRRADIATION

ANNUAL PROGRESS REPORT

JULY 1976

BY

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Exposure of the retina in an awake, task-oriented animal to brief flashes from a Krypton laser produced immediate changes in visual acuity. The magnitude of the initial deficit was dependent upon the size of the exposure on the fovea. The duration of the deficit, on the other hand, was dependent upon the power density of the exposure. The background wavelength of the target had an effect on the derived recovery functions. Exposures to irradiation levels that produced a permanent shift in visual acuity, however, produced an effect somewhat independent of the particular background used to derive spectral acuity.

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INTRODUCTION

Retinal pathology as a result of laser and incoherent irradiation has been extensively studied in the rhesus. Using suprathreshold dosage levels, gross pathological damage has been found to exist in the cornea, pigment epithelium and in the outer segments of the photoreceptors (1-5). As the morphological techniques for detecting minimal retinal alterations have been refined, the threshold power density for such alterations has decreased. Associated with these decreases in exposure power levels is a shift in the site of maximum alteration from the pigment epithelial layer to the outer segments of the photoreceptors. Since the site of anatomical alteration is at the location where the initial transduction of light energy to electrochemical energy occurs, it is important to determine not only the morphological disruptions but also the functional and electrophysiological consequences of the induced alteration as well.

Functional determinations of laser irradiation are of prime concern in determining safety standards for several reasons. First, this approach may provide a more sensitive criteria for determining laser safety thresholds. Minute enzyme changes in the photoreceptors associated with low level laser exposures may not be revealed by more conventional morphological examinations but such alterations may greatly affect the overall functioning of the visual system. Second, morphological criteria alone tell us little about the degree or type of degradation in visual performance. Of course, changes in the ability of observers to perform visually would be of prime concern in missions where successful completion is dependent upon visual and /or visual-motor behavior.

In order to derive the greatest benefit from the laser beam and at the same time develop realistic standards for safety, an accurate assessment of its adverse reaction on the human eye must be carefully examined from both a morphological as well as functional approach. Human experimentation in the area of suprathreshold retinal burns, though, is virtually impossible, since intentional burns can only be performed on eyes that suffer from severe retinopathies or eyes which are slated for early enucleation. Since enucleations are rarely performed until substantial loss of vision has occurred, it is virtually impossible to do complete functional studies on these patients. (6-7). As an alternative, the rhesus has most often been chosen as the experimental model upon which human standards are to be set. The selection of the rhesus as the experimental subject stems from numerous anatomical investigations that have shown that the retinal pathology and physiology of the visual pathways in man and rhesus are quite similar. These facts suggest that the rhesus would make an excellent model for morphological investigations of laser alterations. However, before rhesus behavioral data can be employed in determining the functional consequences of laser exposures in humans, the degree of similarity in visual functioning has to be established for these two species. Since there is a good degree of similarity in structure between the rhesus and human visual system, there is to be expected similarities in function as well. The ability of the rhesus to resolve spatial detail and their sensitivity to color have been shown in the past to resemble that of humans in many ways. Although they are similar, recently some minor differences in the ability of the rhesus to resolve detail under different achromatic luminance conditions have been reported (8). At high luminance levels the acuity of the human observer is superior to that of the rhesus, while the rhesus has slightly better spatial resolution at lower luminance levels. These differences in effective luminances have been attributed to optical rather than to physiological factors. In a previous paper we have reported that the spectral sensitivity of more central regions in the rhesus retina resemble more the sensitivity of the human protanomalous observer than normal human trichromats (9). In these studies spectral sensitivity was measured in both humans and rhesus monkeys using a Landolt ring acuity task with the target being presented in the center of a small screen which the subject was trained to fixate on. Using a chromatic grating pattern which did not require central fixation, Behar and Bock (10) have also reported that the long wavelength sensitivity of the rhesus is reduced relative to that of the normal human observer. Similar evidence suggestive of weak rhesus dichromacy can be observed in the data found in the literature although the magnitude of the long wavelength insensitivity was usually not great and in all cases optical rather than physiological factors have been used to explain this phenomenon. By restricting chromatic visual functioning to the central most regions of the retina rather than presenting targets spread across the entire retinal mosiac, we have represented stronger evidence for photopic protanomalogy which is more difficult to explain purely optically. Such a condition will obviously affect the light absorbing properties of the photoreceptors and, given a thermal model for retinal damage, the tolerance of the retina to long wavelength laser irradiation. Hence, corrections for species differences in terms optical as well as retinal physiology and photochemistry must be made before direct comparisons can be made of the rhesus morphological and functional laser exposure data to predicted human consequences of accidental exposures.

After a correction factor is applied to the rhesus data, minimum functional safety standards can be established for laser irradiation under different laser as well as visual performance conditions. The use of these functional assessments will provide then information not only on the morphological mechanisms but also the adverse effects of laser irradiation on performance criteria of specific missions where eye damage to personnel may occur. This deleterious effect on performance should occur at both threshold and subthreshold power densities for distinct morphological or fundoscopic damage. This functionally oriented injury criteria should also provide a sound base for the understanding of laser eye effects and the establishment of proper guidelines for human eye safety which reflect considerations of the interplay between transitory functional deficits (flash blindness) and the safety and performance criteria of specific missions.

In the past, functional studies concerned with the effects of intense irradiation on the retina have been restricted, however, to the evaluation of severe morphological disruptions of the rhesus fovea or parafovea (1, 12). The effects of these foveal irradiation levels are usually permanent, producing impairments in visual acuity ranging from 40% to 80% of pre-exposure acuity levels. Virtually no exploration of the exposure levels at or below the transition from temporary to permanent visual losses has been conducted, since no technique has been available to expose an awake, task-oriented animal. Immediate acuity effects following intense irradiation are critical, though, in the exploration of thresholds for functional alterations and in determining the consequences of immediate degradation of visual performance on a specific mission. In all previous functional studies, anesthesia was required for placement of the retinal lesions, thereby eliminating the possibility of immediate postexposure acuity measurements for at least 24 hours. The inability to measure transient changes in visual acuity at threshold and subthreshold power levels for permanent alterations, as well as a means to follow the initial phases of the visual deficits elicited by suprathreshold power levels, has been a serious limitation in previous studies dealing with a functional approach to laser safety. In the initial phases of this contractual effort, a procedure was developed for producing consistent foveal exposures in awake, task-oriented animals. This procedure has been used along with a modification of a rapid method to measure rhesus visual acuity to determine the magnitude of the immediate deficit in visual acuity and the temporal characteristics of the recovery process to irradiation levels up to the transition level between temporary and permanent functional alterations (12).

The objectives of the present research program has been to measure the initial deficits in visual acuity and the recovery processes immediately following exposure to laser irradiation. Initial exposures are relatively low in power density and below the threshold for either permanent functional or morphological alterations. The power of the exposure is then systematically increased over a period of time, usually a minimum of six to eight months, until recovery is no longer complete. That is, until recovery to pre-exposure baseline acuity no longer occurs either within the test session or the days which follow. Thresholds for permanent functional deficits are designated at this exposure power level, exposure duration and retinal position. Recovery functions are derived using a continuous assessment of the subject's acuity level immediately prior to and following laser irradiation. This assessment is continued after exposure until total recovery is maintained or, if above threshold for functional alteration, until the recovery process stabilizes. After appropriate corrections are made for species variations, an attempt will be made to extrapolate human visual recovery functions derived under flash blindness conditions (exposures well below the ED50 level and considered safe) to those predicted threshold and suprathreshold energy levels based on the derived data collected in this experiment using rhesus as subjects. The hypothesis is that the curves for recovery in humans and rhesus are similar and are only displaced along the energy dimension due to either differential pigmentation or retinal illumination for the same external physical light source.

In previous protocols, the size of the exposure on the retinal surface had to be restricted to less than 200 microns in diameter due to the power limitations of conventional HeNe laser sources. In the current support period a Krypton laser was used which increased the power output available over standard HeNe sources. This new laser system has allowed for increased exposure diameters on the retina of sufficient power densities to produce functional and morphological alterations while at the same time not significantly shifting the wavelength of the exposing source. This latter consideration allows the previously collected data using a HeNe source to be compared to the data collected with the Krypton line and allows a direct comparison of the effects of the size of retinal area involved with the magnitude and duration of the functional alteration.

The rationale for the use of larger areas of involvement (exposure diameters in excess of 200 microns on the central foveal) is to make the results from these functional explorations more compatible with those of morphological studies. In the latter studies, large areas of involvement were employed to facilitate histological exploration. In addition, the use of larger exposure diameters in our functional studies will allow for histopathological examination of our subjects' retinae by LAIR should that prove beneficial.

METHODS

A detailed description of the paradigm and experimental facilities has been reported elsewhere (9,12). Behavioral assessments of visual performance are measured in a light-tight, primate cubicle isolated from the programming equipment. Two such cubicles are used; one for training and the second for baseline sensitivity measures and exposure to the coherent light source. Both chambers are identical with exception to the addition of optics necessary to present the laser beam coaxial with the discrimination image on the screen. Except for the screen the entire test chamber was dark and all test sessions were preceded by at least 15 minutes of dark adaptation.

A rear projection screen mounted on the far wall of each cubicle subtended 3 degrees at a distance of 1 m from the subject's pupil. Dark Landolt rings against a light background were projected onto the screen using a conventional carousel projector. Background luminance and wavelength were determined by neural density and interference filters placed in the path of the light. Monochromatic backgrounds varied from 420 nm to 700 nm and all filters were calibrated on a Carey spectrophotometer. All monochromatic backgrounds were standardized in terms of quantal irradiance at the 580 nm level. The test patterns were conventional black Landolt rings. The thickness of the rings and the width of the gap that formed the critical detail was always 1/5 of the diameter of the ring. The size of gap was varied from 0.25' to 30' visual angle in 20% steps.

The animal was aligned through a physical restraint device with the center of the viewing screen. The subject's head was kept stationary during testing and exposure by four plexiglas head restraints mounted on the top of a standard primate chair. These restraints temporarily prevented movement in any direction. An opaque facemask and two 5.0 mm monocular iris diaphragms were aligned with the subject's pupils and viewing screen so that eye position could be well controlled during actual exposure to laser irradiation. All testing was done with monocular viewing. A diagram of the optical system is shown in Figure 1

New solid state programming equipment was used to present the appropriate stimulus conditions and to properly reward the animal for his response. This equipment replaced older and less reliable electromechanical programming equipment previously used in this effort. This new equipment has provided for more flexible programming of the experimental parameters and more online data analyses.

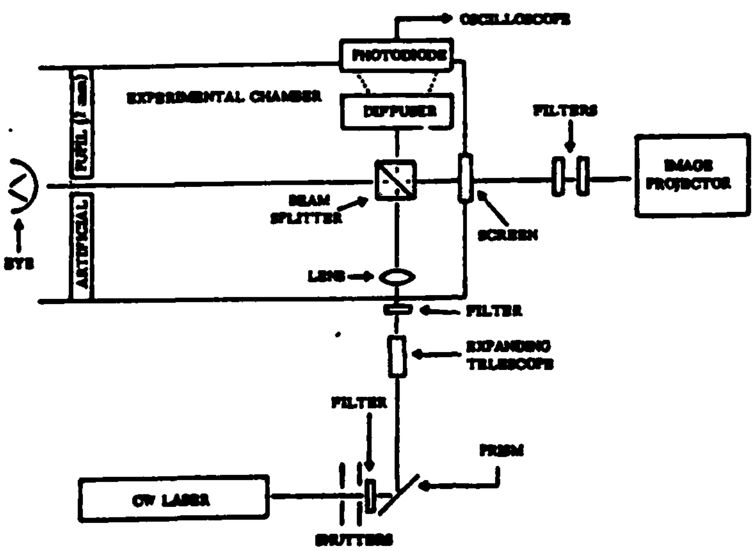

Figure 1. Optical system used to present image targets and the exposing laser irradiation.

<u>Discrimination Task</u>. The subjects were trained, using an avoidance paradigm, to depress a lever whenever a Landolt C was presented. Failure to depress the lever during the 3 second Landolt C trial is punished by a brief electrical shock. Lever responses during trials with completed rings also initiates a brief shock on a fixed ratio reinforcement schedule. The test objects were presented in sets of four rings that were of equal diameter. Three rings in each set were gapless, while the fourth was a Landolt C that appeared in a random position within the set.

Threshold acuity measurements were obtained by an up and down, tracking method which allowed the animal to adjust in discrete steps the size of the test target about his own threshold level. The size of the gap in the Landolt C increased following incorrect "C" responses and decreased by correct "C" detections. Lever responses on completed ring trials did not affect the size of the gap to be presented on the next series of trials. Means and standard deviations of threshold visual acuity were obtained by the use of Dixon and Massey's (13) statistics for the up and down method. During recovery from laser irradiation, the average number of completed rings relative to Landolt C's was reduced from an average of four to two in order to more rapidly track transient changes in visual acuity as a function of time after exposure. Baseline mean levels or variability have not been affected by changes in the ratio of Landolt C's to completed ring trials. Also unaffected were the number of lever responses during completed ring trials which was always very small in our subjects.

Laser System. In the previous projects, a standard 50 HeNe laser served as the adapting light source. During the current period a second 2 W Argon laser with a Krypton plasma tube was used to expose the retina. The major difference between the two sources was the higher output energy of the Krypton laser which allowed for a greater beam expansion for larger exposure sites on the retina (323 microns as opposed to 150 microns) of sufficient power densities to produce a permanent functional deficit. Some slight differences in output wavelength does exist between these two coherent light sources but for our purposes these differences are insignificant.

The entire laser system, with exception of a focusing lens and beam splitter was mounted outside the experimental chamber. The "raw" beam passed first through a manual safety shutter and then a electronic shutter. The shutter was preprogrammed to produced a calibrated exposure duration of 100 msec. The beam was then attenuated by neutral density filters before being diverted by a 4.5 cm diameter front surface mirror. The diverted beam entered a beam expanding telescope which produced a collimated beam of adjustable size. The expanded beam them passed into the experimental chamber and through a 1.25 diopter lens placed 85 cm in front of the subject's pupil. A 5 x 10 cm coated pellicle beam splitter was placed 5 cm in front of the 1.25 diopter lens and at the intersection of the diverging laser beam and the image beam from the carousel projector. Coaxial alignment with the line of sight was verified by noting that the reflected beam also passed through a 2 mm aperture and onto the critical feature of the target on the screen. Mounted on the opposite size of the beam splitter was a diffuser and ultrafast photodiode (HPA-4203). The output of this detector was displayed on a memory oscilloscope and was regularly calibrated against an EFF Model 580 Radiometer placed at the corneal plane. The power and pulse width of each irradiation was measured and recorded. Exposures of 100 msec duration were made at corneal power levels from 1 to 6 mW, beginning with the lowest power level. Retinal energy at 6 mW was no more than 732 mJcm-2, corresponding to a photometric equivalent of 8.32 log td s.

Laser exposure. Prior to any laser exposures, stable baseline acuity levels were established for each subject using both monochromatic and white light targets. Initially, a criterion of, at minimum, 14 consecutive sessions of white light threshold measurements were used to establish a baseline mean and standard deviation for each subject. Following the determination of a stable, white light threshold, the animal's spectral sensitivity (using an acuity criteria) was determined before exposure sessions began. Prior to each exposure, a 15 minute baseline session was completed and the mean for this pre-exposure testing was determined. The number of completed rings relative to incomplete rings (Landolt "C") was then reduced and comparisons made to assure a stable baseline. Failure of the subject to obtain mean acuity within one standard deviation of his pre-determined baseline level on either reinforcement schedule, aborted the session. Session variability which exceeded baseline variability also aborted the session.

Exposures were made during threshold measurements after the above performance criteria were met. The laser flash (100 msec duration) was triggered by the animal's correct detection of his threshold Landolt ring which most often corresponded to gap sizes of between 1.0 and 0.5 minutes of visual angle. The electronic shutter was immediately triggered by a microswitch on the response key. Somewhat causal observations of numerous animals working under these conditions imply that subjects maintain fixation during their response period. The results of this study thus far have shown that triggering the onset of the exposure to the subject's response elicited significant functional deficits in visual characteristic of inactivation of more central regions of the retina. Voluntary eye movements or blink during exposure were eliminated by the use of a 100 msec exposure duration. Exposures were made over power levels increasing in energy from 0.5 mW to greater than 11 mW. No more than one exposure was made per day and exposures were never made either following incorrect detections of Landolt C's or following correct detection during the last 1 second of the trial.

Immediately after exposure, recovery was measured until the subject returned to baseline acuity levels or in the case of a permanent or semipermanent alteration until either the degree of deficit stablized or 45 minutes whichever came first. The session was always terminated after a 15 minute stable was achieved. The entire test session lased approximately 2 hours. If acuity did not recover within a given session, exposure was discontinued on subsequent sessions until evidence of recovery at all spectral points was achieved. The laser exposure level at which this recovery criterion failed to be achieved defined the transition zone between temporary and permanent acuity loss measured in these experiments.

RESULTS

Sample data of threshold acuity using the tracking technique is shown in Figure 2. In this particular session the subject was exposed to a 7 mW, 150 micron HeNe flash of 100 msec duration. Similar data was observed in the present study using the Krypton laser and a larger, 323 micron spot on the retina although the magnitude of the initial deficit was typically larger when a larger portion of the retinal mosiac was involved. The occurence of the exposure is indicated in this figure by an arrow (zero point on the abscissa). The ordinate indicates the various sizes of the gaps in presented Landolt rings and is plotted in reciprocal minutes of arc. The order of presentation was dependent upon the subject's response on Landolt ring trials. Incorrect detection of the Landolt C caused the recorder to plot downward and corresponded to the presentation of larger ring diameters. The abscissa represents the presentation of the Landolt Cs, and corresponding times (in minutes) for representative trials are indicated relative to exposure.

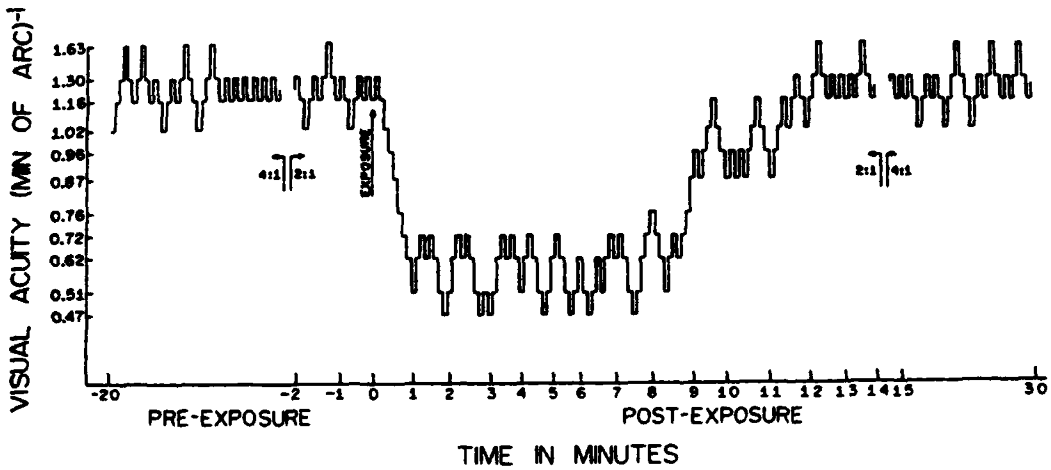

Figure 2. Sample data of threshold acuity using a tracking technique prior to and immediately following laser irradiation.

Prior to exposure, the subject's mean acuity under maximum photopic conditions was 1.25 (min of arc)-1. Immediately following exposure the subject's acuity to achromatic targets decreased 59% to an acuity level of 0.51 (min of arc)-1 and full recovery was complete within 13 minutes. With an expanded beam diameter exposing a greater portion of the central retina, larger initials deficits were observed. The magnitude of the initial deficit was independent of exposure power and with retinal exposures of 323 microns the initial deficits ranged from 60% to 80% of the pre-exposure baseline level. A comparison of the recovery functions for two different degrees of retinal involvement, together with a control or sham exposure condition, is shown in Figure 3. The "no exposure" or sham condition represents the time for the animal to return to baseline following an artificial increase in the size of the targets to near the acuity level typically immediately observed following exposure to a 150 micron spot. This time represents the finite time for the program to reverse itself and track backwards as the animal correctly detects suprathreshold targets. For equal energy exposures of different beam diameters, a significantly larger initial deficit was noted for the larger exposure spot. Exposure power had little or no effect on the magnitude of this initial deficit. The duration of the total recovery function was, however, strongly influence by exposure power and only moderately affected by beam diameter. In the present example, total recovery time for equal exposure power densities (1 mW) were the same in spite of a difference in total power and area of involvement.

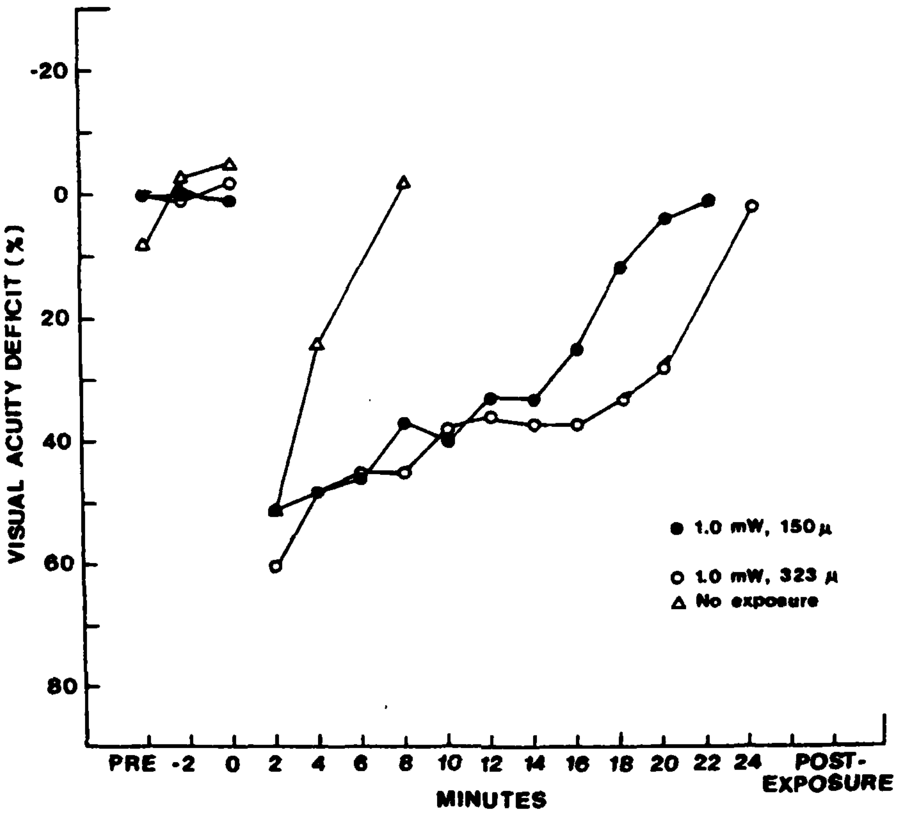

Figure 3. Comparisons of the recovery functions for two different diameter exposures on the retina.

As mentioned previously, since the area of involvement was the fovea, perhaps chromatic as opposed to achromatic acuity targets would be more appropriate in determining the degree of visual deficit and the time course for any recovery which might be possible following laser irradiation. In Figure 4, recovery functions following three separate 6.0 mW exposures to a Krypton laser producing a 323 micron spot on the retina are shown. All chromatic background for the test targets were equated for equal numbers of quanta. The largest observed initial deficit was plotted using a 560 nm background while the magnitude of the initial deficits for both 480 nm and 640 nm were similar.

Overall the shape and total time course of the recovery functions were similar. In order to determine any statistically significance between these functions plotted using different monochromatic background more data is needed. What appeared significant were the differences between the use of monochromatic and achromatic backgrounds to follow the time course of foveal recovery following laser irradiation. Typically longer and larger deficits were observed when monochromatic backgrounds were used to follow exposures similar diameter and power densities on the retina.

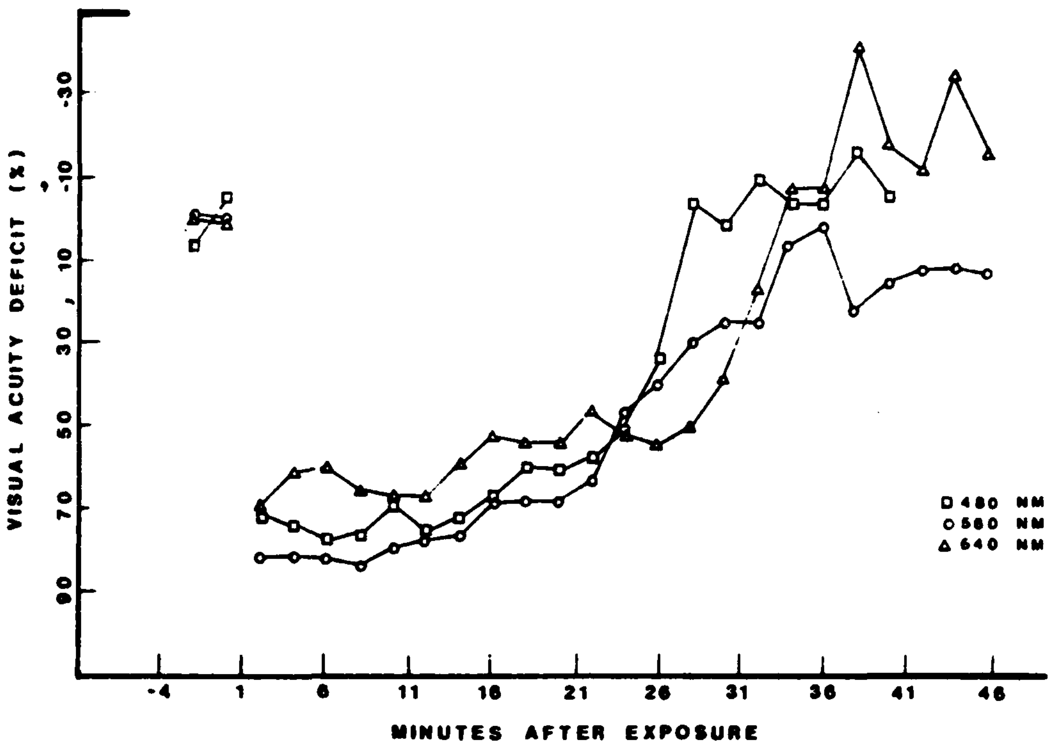

Figure 4. Visual acuity deficit (%) relative to pre-exposure acuity following three separate 6.0 mW, 647 nm exposures. In each case a different background wavelength was used to follow the deficit in visual performance.

In one animal, a significant shift in chromatic acuity was observed following repeated exposures to 6.0 mW, 647 nm light. Only one exposure was presented each day and typically recovery was complete by the end of testing session for all but the last exposure. The postexposure acuity presented in Figure 5 represent the maximum acuity of the animal two weeks after the laser exposure session. To both chromatic and achromatic acuity, this animal showed a significantly decreased acuity in the exposed eye between the pre- and postexposure condition. No significant shifts in pre- and postexposure acuity was noted in the control (unexposed) eye during this same time period. Similar to the chromatic acuity, achromatic acuity was also depressed during this same time period.

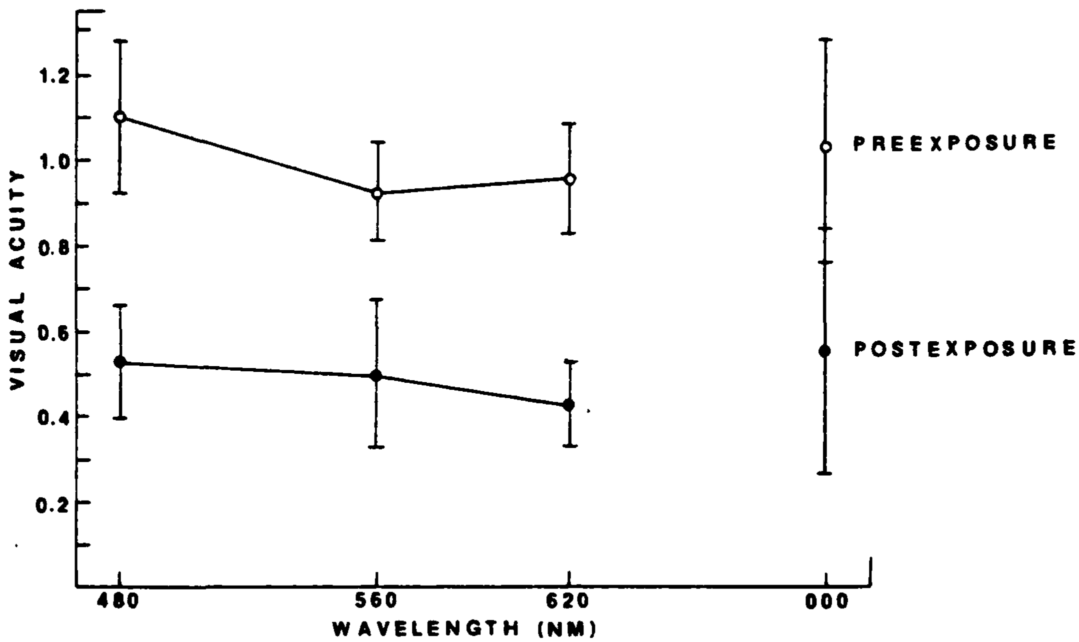

FIGURE 5 Pre- and postexposure chromatic acuity following a long term shift in baseline acuity after laser irradiation.

DISCUSSION

An advantage of the employment of larger exposures sites on the retina has been to increase the probability of a foveal exposure in any given session since a larger retinal area will be affected. In past studies we have not found that the subjects typically could "look" around the affected retinal area and still make the baseline "photopic" discriminations. If a deficit was elicited by an exposure, both the magnitude and duration of the deficit suggested the total foveal involvement occurred and any attempt to "look" around the affected area resulted not in maximum visual acuity but rather acuity levels more characteristic of parafoveal vision. In previous studies, however, a number of exposures produced no deficit suggesting that the subject either blinked or moved his eye slightly preceding the exposure so that the beam entered slightly off the central axis. In these cases, the subject could possibly still "look" around the affected area and still make baseline maximum photopic discriminations since the entire fovea was not involved. In the present study with larger areas of involvement, the percentage of these "misses" of the central fovea has decreased significantly.

Increasing the spot size of the exposing source also has had some methodological significance in regard to the degree of the deficit elicited. In the original program using smaller diameter exposures, a deficit of 20/40 was consistently elicited regardless of exposure power and the magnitude of this deficit was interpreted as the maximum acuity of the surrounding parafoveal area outside the central 150 microns of the foveala. This size deficit was produced regardless of the power density of the exposure up to and including those energies which produced a permanent functional alteration in visual acuity. An acuity level of 20/40 does correspond nicely to parafoveal vision and is suggestive of total foveal involvement. Larger affected areas employed in the current study not only inactivated the entire foveal region but also larger areas of the surrounding parafovea and did produced significantly larger deficits. Acuity is not uniform across the retinal mosiac and decreases as one progresses more off-axis. As expected the larger diameter exposure sites produced an initial deficit larger in magnitude than did smaller diameter sites. The magnitude of the change in the initial deficit was smaller than might be expected from almost doubling the area of retinal involvement. This is to be expected though, since the fall-off in acuity outside the central fovea is rather shallow at first as one proceeds off-center. Continued exposures with larger diameter spots in subsequent studies will provide additional data which can be statistical compared with the data collected using smaller diameter spots in previous studies.

In the past, recovery functions from laser irradiation were derived using only white light test backgrounds of a single intensity and contrast level. These test conditions elicited maximum photopic acuity from the animal. Since the area of primary disruption was the fovea monochromatic as well as achromatic targets would appear appropriate test conditions. During this period we have begun examining recovery using monochromatic test conditions in addition to the conventional achromatic targets and found some interesting differences in the nature of the recovery function when using different background targets. Because of the increased number of test parameters employed to examined both chromatic and achromatic recovery, this phase of the project was not completed during the current support period and will be reported in more detail in future reports.

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